STUDIES IN THE GENUS *UROMYCLADIUM* (UREDINEAE). I.

GENERAL INTRODUCTION, THE ANATOMY OF THE GALLS, AND THE CYTOLOGY OF THE VEGETATIVE MYCELIUM AND PYCNIA OF UROMYCLADIUM TEPPERIANUM (SACC.) MCALP. ON ACACIA STRICTA WILLD.

By Alan Burges, M.Sc.,
Linnean Macleay Fellow of the Society in Botany.

(Twenty-four Text-figures.)

[Read 25th July, 1934.]

Introduction.

In Australia, work on the rusts has been more or less limited to species of economic importance—principally *Puccinia graminis*—and to systematic problems. The cytological details of almost all our indigenous rusts are unknown. This fact, coupled with the interest stimulated by recent research on the functions of the pycnium in rusts, led to the present study of a common and essentially Australian genus.

The genus *Uromycladium* (McAlpine, 1905) consists of seven species, *Ur. simplex* McAlp., *Ur. Robinsoni* McAlp., *Ur. maritimum* McAlp., *Ur. alpinum* McAlp., *Ur. notabile* (Ludw.) McAlp., and *Ur. Tepperianum* (Sacc.) McAlp., of which *Ur. simplex* is the type species for the genus. Arthur (1906) divided the above into two genera, calling his new genus *Macalpinia* and taking *Ur. Tepperianum* as the type. He retained the name *Uromycladium* for the other. Clements and Shear (1931) do not recognize the validity of this division, and most mycologists agree.

Six species are endemic to Australia and the seventh, *Ur. Tepperianum*, is reported to extend to Java (Magnus, 1892). The six endemic species, so far as is known at present, are restricted to the genus *Acacia*, but *Ur. Tepperianum* occurs on *Albizzia montana* as well (Magnus, 1892). Two species, *Ur. Tepperianum* and *Ur. notabile*, are of particular interest because they form large galls on the branches and phyllodes of their hosts.

The systematic position of the genus *Uromycladium* is in the Raveneliae section of the family Pucciniaceae (Arthur, 1929, p. 144). The genus forms a well-marked group characterized by the presence of a pedicellate, depressed globose teleutospore, and the occurrence of more than one spore on the same stalk. Apparently all the species are microcyclic with the possible exception of *Ur. bisporum*, which is incompletely known, the teleutosporic stage being the only one so far recorded.

Ur. simplex and *Ur. Robinsoni* (Gaumann and Dodge, 1928) are considered to be closely allied to Uromyces, but are distinguished by the presence of the characteristic depressed globose teleutospore and the cyst. In *Ur. bisporum* two teleutospores occur, but the cyst is absent. *Ur. maritimum* and *Ur. alpinum* have

two teleutospores and a cyst. The two remaining species, *Ur. notabile* and *Ur. Tepperianum*, lack the cyst but have three teleutospores in a head.

The cyst when present is usually derived from the basal cell and is generally considered as being comparable with the cyst of *Ravenelia* (Gaumann & Dodge, 1928, p. 579). From a study of the segmentation of the spore groups in various species, it appears evident that the cyst is not a special structure, but is morphologically a teleutospore. This conclusion is supported by the occasional occurrence of a perfect spore in the place of the cyst (*vide* McAlpine, 1906, Plate xxi, fig. 179).

In *Ur. Tepperianum* the three teleutospores are formed in the manner indicated in Figure 1, *e* and *f*. This figure also illustrates the method of segmentation of the two spores and the cyst in *Ur. maritimum* (Fig. 1, *a–d*). The nature of the divisions suggests that *Ur. maritimum* is derived from a type such as *Ur. Tepperianum*, at least so far as the teleutospore structure is concerned. A similar suggestion may be advanced regarding *Ur. bisporum* (Fig. 1, *g* and *h*) and *Ur. simplex* (see Fig. 1, *g* and *i*). From a consideration of the known facts, the derivation of a Uromycladium from a Uromyces type might have proceeded along some such lines as these: the first step would be the duplication of the teleutospore giving rise to the condition met in *Ur. bisporum*. Specialization of one of the spores might lead to the formation of a cyst and produce the *Ur. simplex* type. If, instead of forming two spores in the head, three were developed, a similar modification of a spore to a cyst would give the *Ur. maritimum* type from the *Ur. Tepperianum* type (cf. Fig. 2).

Ravenelia may be considered as the climax of such a series. The spore head of *Ravenelia* could be interpreted as a fascicle of *Uromycladium* teleutospores each with a cyst attached. The fusion of the individual portions to form the head is secondary.

Although originally the species of *Uromycladium* were limited to Australia and Java, some have attained a much wider range. Introduction of a host plant (*Acacia decurrens*) into South Africa and New Zealand has resulted in the spread of *Uromycladium Tepperianum* in both countries. During a stay in New Zealand in December and January, 1932-33, specimens were collected from numerous localities. In every place visited in which trees of *Acacia decurrens* were growing, some were found to be infected with *Ur. Tepperianum*. At Nelson and at Auckland the infections were heavy. In South Africa the disease has spread to many of the Wattle Bark plantations and, as in Victoria, causes severe damage to the trees.

Ur. Tepperianum has by far the most extensive host range of the genus. McAlpine, in 1906, recorded it from nineteen species of *Acacia*. Since then the knowledge of the host range has been considerably extended and now includes the following forty-nine species:

aneura F.v.M., *armata* R.Br., *auriculaeformis* A. Cunn., *Baileyana* F.v.M., *binervata* D.C., *bynoeana* Benth., *calamifolia* Sweet, *dallachiana* F.v.M., *dealbata* F.v.M., *decurrens* Willd. and vars., *diffusa* Edw., *elata* A. Cunn., *erioclada* Benth., *fasciculifera* F.v.M., *glaucoptera* Benth., *glaucescens* Willd., *hakeoides* Cunn., *holosericea* A. Cunn., *homalophylla* A. Cunn., *implexa* Benth., *juniperina* Willd. and vars., *linifolia* Willd., *linophylla* Fitz., *ligulata* A. Cunn., *longifolia* Willd., *Maideni* F.v.M., *melanoxylon* R.Br., *microbotrya* Benth., *montana* Benth., *myrtifolia* Willd., *neriifolia* A. Cunn., *notabilis* F.v.M., *obliqua* A. Cunn., *Oswaldi* F.v.M.,

penninervis A. Cunn., *pruinosa* A. Cunn., *pycnantha* Benth., *retinoides* Schlecht., *rigens* A. Cunn., *salicina* Lindl., *siculaeformis* A. Cunn., *spinescens* Benth., *stricta* Willd., *tetragonophylla* F.v.M., *trinervata* Sieb., *trineura* F.v.M., *verniciflua* A. Cunn., *verticillata* Willd., *vomeriformis* A. Cunn.

So wide a host range as this, including as it does species from all sections of the genus *Acacia*, naturally raises the question as to whether *Uromycladium Tepperianum* is only one species or a group of closely related physiological strains. The latter idea seems more acceptable. Samuel (1924) states that "This great range of infection of *Ur. Tepperianum* cannot but raise doubts as to the physiological homogeneity of this species. Field observations of the fungus only increase these doubts." Before making the above comment Samuel had remarked on the wide divergence of gall types and the occurrence in some cases of witches' brooms, varying forms being associated with different species of hosts. At Ooldea (S. Australia) Samuel (1924) states that *Acacia ligulata* and *A. linophylla* are to be found growing together in many places. In one definite area *A. ligulata* was heavily infected and *A. linophylla* free, while in another area the reverse was the case. To quote further from Samuel, "This cannot but suggest that the fungus affecting one is not cross-inoculable to the other, although morphologically the two fungi are identical in every respect, and referred to the one species *Uromycladium Tepperianum*." He finds a similar state of things between plants of *A. armata* and *A. pycnantha*.

These observations are borne out by those made by the present writer, in New South Wales. At Pennant Hills (N.S.W.) clumps of *Acacia stricta* have been seen growing together with *A. Maideni*.

The former species was heavily infected and the latter quite free of disease. About half a mile away the reverse was the case. Similar observations have been made of *A. stricta* and *A. juniperina*, and *A. stricta* and *A. myrtifolia*.

So far as is known at present, all the species of *Uromycladium* are microcyclic. In the three species examined, *Ur. Tepperanium*, *Ur. notabile* and *Ur. maritimum*, the vegetative mycelium is uninucleate and gives rise to pycnia. The binucleate mycelium is not extensive and is limited to the spore-producing layer. This forms uredospores and then teleutospores or, in the more reduced forms, only teleutospores. Subsequent reinfection is by uredospores or sporidia. The origin of the binucleate condition is still in doubt. Definite examples of nuclear migration have been found in the hyphae which form the developing sorus, but the paucity of examples suggests that the method may not be the chief means of the initiation of the diploid phase. During a study of the pycnial stage, hyphae have been observed projecting from the hymenial layer, and in some cases these have been shown to be binucleate. Further, it is possible to follow a line of hyphae which stain more readily with saffranin from some old pycnia to the developing teleutosorus. In a few cases the teleutosorus has been shown to develop at the base of the pycnium. The matter is still being investigated.

Gall Structure.

Although the ability of fungi to cause the formation of galls is not rare, it is, nevertheless, limited to comparatively few genera which are widely separated systematically. Among the rusts, *Gymnosporangium* (Sandford, 1888) and *Cronartium* (Dodge and Adams, 1918) are well known examples of gall-forming fungi, and some species of *Puccinia* (e.g., *P. rubigo vera* on *Clematis*) possess the same capacity. None of these, however, produces galls comparable in size to

those caused by *Uromycladium Tepperianum*. McAlpine has recorded galls on *Acacia implexa* weighing as much as three pounds, and the writer has observed some, not far short of this weight, on the same species at Canberra (F.C.T.).

Infection by *Uromycladium* causes different results on different hosts and occasionally the reaction of the same host species may vary in different habitats. At National Park (N.S.W.) plants of *Acacia myrtifolia* were found infected with *Ur. Tepperianum*. This had led to the formation of galls about 1–2 cm. in diameter. The conditions under which the host plants were growing were favourable—well-drained soil and ample water supply. Specimens of the same host infected with the same fungus but collected from Central Australia (Macdonnell Ranges) showed no gall formation. The fungus caused the bark to be ruptured and filled the opening with a dense layer of teleutospores. Apparently the more severe conditions of the Central Australian habitat prevented the usual hyperplasy of the stem tissue.

Of the two gall-forming species of *Uromycladium*, *Ur. Tepperianum* is more widespread and shows a greater variety of gall forms than does *Ur. notabile*.

When the infection by *Ur. Tepperianum* is confined to the phyllode, the gall is small, seldom more than 0·5 cm. in diameter. It is on stems that the greatest distortion occurs. On *Acacia juniperina* the stem galls are spindle-shaped, tending to be irregular and from 1 to 5 cm. long. Usually they are not more than 0·75 cm. wide. Normally on this species the gall is annual, but now and then odd perennial galls are found. The galls on *A. stricta* are larger than those on *A. juniperina* and may be as much as 8 or 9 cm. in length and 3–4 cm. in breadth. The gall is normally annual, but the evidence seems to indicate that there is a tendency for the mycelium to perennate in the stem tissues. The foregoing species of *Acacia* are shrubs; when trees become infected, as in *A. implexa* or *A. glaucescens*, the galls are usually perennial and may have a life as long as eight or ten years. The continued growth produces the large galls referred to earlier in the paper. In most cases these perennial galls do not produce spores continually, but undergo a resting period, usually in the dry months of the year. This resting period is by no means well defined and different galls vary considerably in their activity.

As material of *Acacia stricta* infected with *Ur. Tepperianum* was available in large quantities near Sydney, the galls on this species were used for the anatomical study.

The host plant is a shrub or sometimes a small tree which grows to about 15 feet in height. True leaves are absent in the adult plant, their place being taken by phyllodes which are vertically flattened. The inflorescence is axillary, the flower heads being either solitary or in pairs on short peduncles.

As *A. stricta* frequently occurs in almost pure societies in the field, infection spreads readily. No part of the plant above the ground is immune from infection. Phyllodes, stems and branches, inflorescence and pods may all be diseased. The axillary shoot is particularly susceptible—probably because spores can lodge in the axil and are thus more or less protected.

The first evidence of infection is visible, as a rule, in February or March. If a young inflorescence shoot is concerned it may become completely distorted and hypertrophied in a few weeks. Infection on stems or phyllodes does not lead to such rapid distortion. On the stem the swelling of the tissue causes an early bursting of the epidermis or bark, according to the age of the organ concerned. The gall tissue which swells through the fissure is fairly smooth on

the outer surface. At this stage pycnia are formed most abundantly. Towards the end of March teleutospores make their appearance in large numbers. Growth of the galls is rapid and by April or May they are about 3–4 cm. long and 1–2 cm. in diameter. The confluence of the developing teleutosori soon covers the whole gall with a rusty mass of spores. Spore production is continued throughout most of the winter. Towards August the galls begin to die and spore production ceases. When the galls are on thick branches they are perennial occasionally, and in the following year produce warty out-growths which bear pycnia and teleutosori as before.

Anatomy.

The stems of *Acacia stricta* are ribbed, partly owing to the tendency for the phyllodes to be decurrent. Normally there are about five ribs. A section of a stem is represented diagrammatically in Figure 3. The young stems are bounded by an epidermis (*a*) which is very slightly papillate. The cuticle is very thick and tends to emphasize the slightly papillate nature of the epidermis. Beneath the epidermis is a layer of collenchyma (*b*) several cells in thickness. The cortex is very narrow. Pericyclic fibres (*c*) are well developed and may form a complete ring. The phloem area (*e*) is extensive for a stem and contains numerous islands of fibres (*d*). The cambial zone (*f*) is very narrow and poorly defined, and frequently indented: these indentations do not, however, coincide with the grooves on the stem. Secondary wood (*g*) is extensive and shows annual rings. Vessels (*k*) are few in number—fibres and tracheids composing the greater part of the xylem. There is little wood parenchyma. Medullary rays (*h*) are well marked and are simple. Starch is present in the xylem fibres surrounding the vessels and in the pith cells (*i*), these latter being simple pitted parenchymatous units.

Infection occurs generally before cork formation has begun, but in a few cases the fungus may force an entry through the cork-bound stem. Abnormalities in the stem are first visible in the phloem where the cells enlarge and resemble parenchyma. Small patches of cells become meristematic and rapidly cause distortion of the outer cells, and eventually rupture of the cortex and epidermis. At this stage few hyphae and haustoria are visible. About the same time as the epidermis breaks, the cambium, and with it the young xylem, are infected. During this period large numbers of haustoria appear in the phloem region. In the early stage of xylem disturbance the fungus is not much in evidence, but it alters the physiology of the plant sufficiently to prevent lignification of the newly-formed xylem elements. At the same time large quantities of plastic substances, which appear to be of the nature of tannins, are deposited in the outer cells of the developing gall. When the fungus is well developed and has produced haustoria in most of the unthickened cells no more plastic substances are deposited. Subsequent development of the fungus causes the throwing off of the outer layers (see p. 218 and fig. 5) so that only little tannin may be present in a well developed gall.

In view of Wood's (1932) work on the correlation of the disappearance of tannins with the increase of lignification, it is tempting to suggest that the tannic material would normally go to the lignification of the xylem, but owing to the disturbance of the normal physiological conditions of the cell has been diverted. As the fungus at this stage is not developed extensively, the amount of food material is in excess of its requirements, and this excess is deposited

in the outer cells. When the fungus is fully developed, and sporing freely, most of the available food material is used, and little or no tannin occurs.

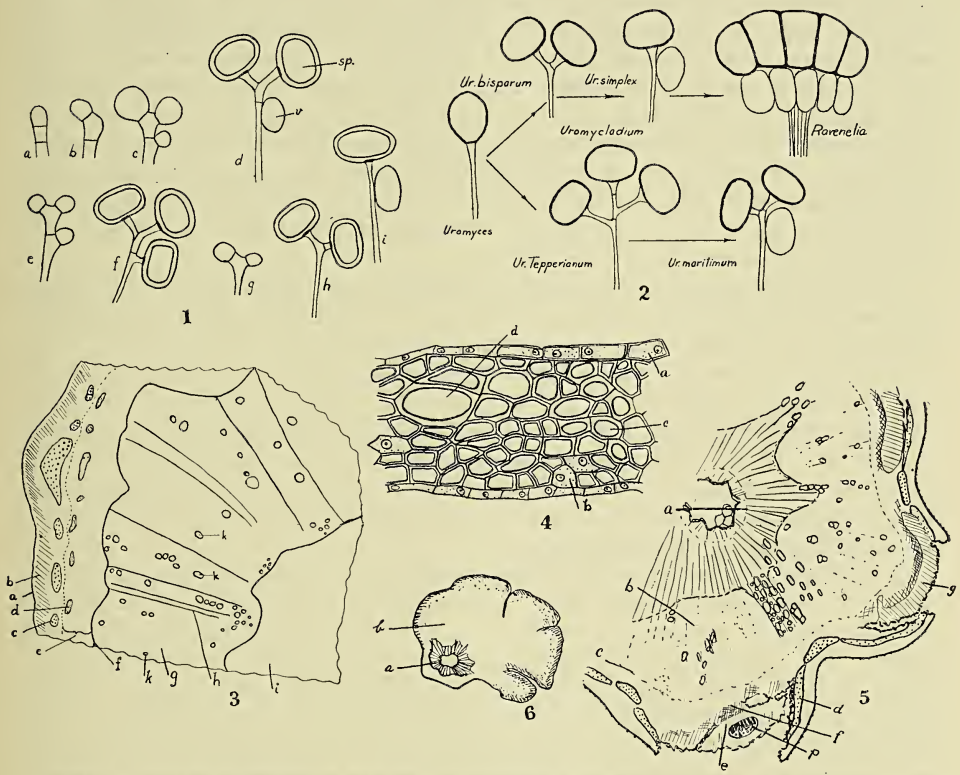

Fig. 1.—*a-d*, segmentation of the teleutcspores of *Uromycladium maritimum*; *e-f*, *Ur. Tepperianum*; *g-h*, *Ur. bisporum*; *g* and *i*, *Ur. simplex*; *sp*, spore; *v*, vesicle. All diagrammatic.

Fig. 2.—Diagram showing suggested lines of evolution.

Fig. 3.—Camera lucida drawing of a portion of a normal stem of *Acacia stricta* in cross section. *a*, epidermis; *b*, collenchyma; *c*, pericyclic fibres; *d*, phloem fibres; *e*, phloem region; *f*, cambial zone; *i*, pith; *k*, vessel. × 17.

Fig. 4.—Normal xylem from *Acacia stricta* stem. *a*, cell of medullary ray; *b*, wood parenchyma; *c*, fibre; *d*, vessel. × 333.

Fig. 5.—Diagrammatic section of a young gall caused by *Uromycladium Tepperianum* on *Acacia stricta*. *a*, normal xylem; *b*, affected xylem; *c*, position of the cambium; *d*, pericyclic fibres; *e*, portion of gall being exfoliated by *f*, developing teleutosorus; *g*, exposed hyperplasied phloem; *p*, old pycnium being thrown off by developing teleutosorus. × 10.

Fig. 6.—Cross-section of an old gall on *Acacia stricta*. *a*, normal xylem; *b*, gall tissue. × 1.

In the phloem regions the disturbance caused by the fungus is due to an increase in size of the cells and a return to the active conditions. A similar state of affairs occurs in the xylem. Even in young galls it is very difficult or even impossible to locate the cambium. The normal radial arrangement of the xylem is recognizable in the young galls, the cell rows being continuous with those formed prior to hyperplasy, but in the older galls irregular division of the

still active xylem elements makes the identification of tissues extremely difficult. The occurrence of a few tracheids here and there helps somewhat, but, as Butler (1930) points out in his study of the morbid anatomy of plant galls, most cells have the capacity to develop in any direction, and perfect tracheids may be formed from cortex or other tissues. Figures 3–8 illustrate the structure of the uninfected and infected tissue. Figure 3 is a camera lucida outline drawing of a portion of a normal stem of *Acacia stricta*. The details of the xylem are shown in Figure 4. In Figure 5 the structure of the young gall is shown diagrammatically. At *g* (Fig. 5) the epidermis and cortex have been ruptured by the hyperplasia of the phloem which is extruding through the break. The exfoliation of the outer layer at (*e*) is due to a developing teleutosorus (*f*). This also causes the old pycnia (*p*) to be shed.

In an old gall the outer surface is irregular, owing to the activity of isolated meristematic zones (see Figure 8) in place of the normal continuous cambium. Figure 6 is a drawing of a cross-section of an old gall.

Mention has been already made of the failure of the greater part of the affected xylem to lignify. In the earlier stages (Fig. 7) the structure of the xylem is abnormal only in the lack of thickened lignified walls on the cells. Medullary rays can still be detected (*a*, Fig. 7), and well-formed vessels or tracheids are present. As the gall continues to grow, isolated patches of cells become meristematic (Fig. 8), and by their growth they crush and eventually cause exfoliation of the outer layers.

The length of life of the cells forming the gall is variable. Usually the fungus does not seem to cause rapid death. At the time when the cells of the gall are dividing rapidly, intercellular mycelium is very scarce and haustoria are almost absent. The intensity of the stimulus of the rust is shown by the marked activity of the host in comparison with the small amount of parasitic growth. Usually in August or September weather conditions limit the further growth of the fungus, and with it the gall. Following the death of the parasite many of the gall cells become lignified to form short tracheids or sclereids, and the remaining cells of the gall die. In most old galls insects are common, and these secondary invaders frequently terminate the life of the gall earlier than might have otherwise been the case.

In addition to insect invaders, other fungi are frequently present. Some of these are definitely parasitic, others appear to be saprophytes or weak parasites. *Darluca filum*, which attacks the *Uromycladium*, may frequently reduce a spore output to half, or less than half of the usual number. Other types appear to be parasitic on the gall tissues, such as *Pestolozzia* sp., *Macrosporium* sp., and an undetermined Ascomycete belonging to the Sphaeriales. The pycnial fluid, especially in moist weather, provides a medium for the development of *Cladosporium* and *Penicillium*. Odd members of the Imperfectae appear from time to time, but are never important.

Comparison with other Galls.

Butler (1930), in his study of the morbid anatomy of plants, divided the various kinds of galls into groups according to the various regions hypertrophied. He classes the Acacia rust-gall as one involving both the cortex and the vascular cylinder. The Acacia gall examined by Butler was caused by *Ur. notabile* on *Acacia decurrens*. On this species, the fungus usually attacks the rachis of the bipinnate leaf; but, apart from this difference, there appears to be little variation

between the sequence of tissues attacked in both this species and *A. stricta*, with perhaps one notable difference. Butler records that phloem hypertrophy is extensive and that xylem hypertrophy may not keep pace with it and may come to an end fairly soon. In the galls on *A. stricta* the reverse appears to be the case, for nearly all the gall tissue is made up of deformed xylem. Where fungus galls are formed from the vascular cylinder they are usually woody, as in *Gymnosporangium* (Harshberger, 1902), and there is little or no trace of delignification. Sometimes in *Gymnosporangium* arcs or sectors of the wood consist of parenchyma—representing unlignified xylem (Wornle, 1894). There are, however, fungal galls which show similar features to those of *Uromycladium Tepperianum* on *Acacia stricta*. For example, in the witches' broom of Cocoa (*Marasmis perniceosis*) the gall is composed of all the stem tissues (Went, 1904) and hypertrophy exists in the xylem, the affected cells of which are largely parenchymatous. In the bacterial galls of *Rubus occidentalis* the gall wood is sharply differentiated from the normal wood by the scarcity of fibres, and the thinner walls and larger lumina of the cells. Isolated patches of tracheids are also present (Butler, 1930, p. 192). Thus the prevention of lignification is by no means confined to the galls of *Ur. Tepperianum*, being possessed in varying degrees by other organisms. The occurrence of well-formed tracheids in the centre of parenchyma is seen in *Gymnosporangium* (Stewart, 1915).

In many types of fungal infection there is an attempt on the part of the host to isolate the infected region by bark formation. Apparently the balance between the host and parasite in the *Uromycladium* gall is very delicate, as is the case in most rusts, and the reaction of the host is such that no cork formation is initiated in the region of fungal infection.

Morphology and Cytology of Uromycladium Tepperianum.

As in the case of the examination of the gall structure, *Acacia stricta* infected with *Uromycladium Tepperianum* was the most convenient source of material for the study of the morphology and cytology of the fungus.

Most of the work was done by means of microtomed sections. Various fixatives were used, including Carnoy, Bouins, Formal-Acetic-Alcohol, Chrom-acetic and Flemming's Special Fixative. Of these Flemming's Special Fixative and Chrom-acetic Fixative gave the best results. Sections of varying thickness were cut; for studying nuclear details of the pycnia 2μ sections were preferred, but for haustoria, sections cut at 10μ were better. Most of the sections were cut at 4μ.

When staining, Iron Alum Haematoxylin, Flemming's Triple stain and Newton's Gentian Violet were used most frequently. The haematoxylin gave the best preparations of nuclear division in the pycnia, but Flemming's Triple was much to be preferred when working with the teleutosorus and teleutospores. Wherever possible results obtained with Iron Alum Haematoxylin were checked by a comparison of material stained with a transparent stain such as Flemming's Triple or Newton's Gentian Violet. For a general study of the distribution of the mycelium Thionin and Orange G proved useful, and for staining fresh material, Acid Fuchsin was employed. Congo Red was found to be an excellent stain for the fungal cell walls, but in thick sections it tended to obscure detail.

The Vegetative Mycelium.

The entrance of the sporidial germ tube and the early stages of infection have not yet been observed. Apparently no part of the host plant above ground is

immune. Frequently each shoot on a branch is infected, including the developing inflorescences. In such a case the great extent of infection may have been due either to a heavy dusting of spores or to progressive infection of the branch, starting from a single infection. This latter view seems the less likely. Careful staining seldom reveals hyphae at any great distance from the galls, indicating that *Uromycladium*, like most rusts, has a fairly limited mycelium which is localized at the seat of infection. The period of incubation is not yet known, but evidently it may be as long as months. Spore shedding is at a maximum in May, June and July, but the new crop of galls seldom appears before February. Once the galls have commenced growth, weather conditions appear to have little effect on them except that when they are mature, dry weather may completely check spore formation.

In the youngest cases of infection examined, the haustoria and most of the mycelium are confined to the cortex and phloem. Mycelium and haustoria do not appear in the wood to any marked extent, until fairly large areas of modified xylem are to be found.

The mycelium is intercellular but forms intracellular haustoria. Generally the hyphae are about 3μ in diameter, although occasional examples may reach 5μ. Frequently several strands lie in the same intercellular cavity, which becomes greatly extended by the growth of the fungus. Septa are frequent throughout the mycelium, giving the individual cells the short stumpy appearance generally associated with rust mycelia. The thin hyphal walls conform to the shape of the host cell-walls bounding the intercellular spaces, so that the rust cells are often slightly irregular in outline. Each cell contains a single nucleus, finely granular cytoplasm and usually one or more vacuoles. The number of vacuoles found varies. In actively-growing hyphae none or only small ones are present. In the hyphae distant from the sorus at the time of sporing the number of vacuoles increases, and the individuals themselves enlarge. Towards the end of the sporing period most of the mycelium is extensively vacuolated. Usually small granules can be detected in the hyphae but the nature of these is unknown. In living material oil drops have been seen in the hyphae but their occurrence is not extensive. The nuclei are comparatively large and usually centrally placed in the cell. There is a definite nuclear membrane, a single nucleolus and chromatin material which is in the form of an irregular network lining about one-half to two-thirds of the nuclear membrane. In most nuclei there is a darker staining body in the centre of the network, and this may correspond to a centrosome. A similar arrangement of chromatin material in the rust nucleus is recorded by Colley (1918), Olive (1908), and others.

Haustoria are produced during the early stages of formation of the gall. Magnus (1900), however, records that in *Puccinia leucosperma* the haustoria arise late, and considers that the main nourishment is obtained by the osmotic activity of the intercellular mycelium. The early occurrence and the number of the haustoria in *Uromycladium* seem to indicate that the amount of food material absorbed through the host wall is probably small. Any part of a hypha may produce a haustorium. The young haustorium seems to penetrate the host cell wall without difficulty and there is no attempt on the part of the host to thicken the wall at the point of entry, although there is always a definite constriction of the hypha as it enters the host cell. Once having penetrated the cell wall, the hypha comes in contact with the primordial utricle. The subsequent

development in other rusts has been variously interpreted, but the general consensus of opinion is summed up by Arthur (1929, p. 117): "The plasma membrane of the host cell is not at first penetrated by the haustorium, but on the contrary appears to be merely invaginated and pushed inwards by the growing tip. It is probable that in no instance does the haustorium come into organic contact with the protoplasm of the host cell."

Young haustoria have been examined in *Uromycladium* but no evidence of invagination of the primordial utricle has been found. Colley (1918), in describing the haustoria of *Cronartium*, states that no invagination occurs; also,

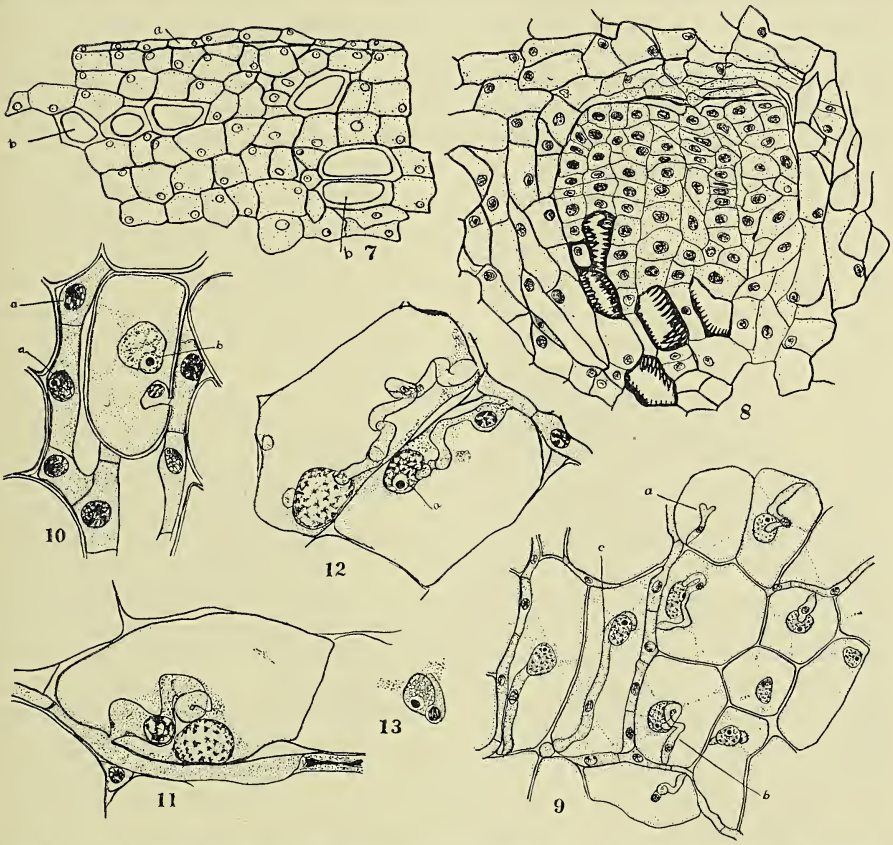

Fig. 7.—Affected xylem from gall on *Acacia stricta*. *a*, medullary ray; *b*, vessel; *c*, fibre or tracheid. × 333.

Fig. 8.—Isolated meristematic zone in gall of *Acacia stricta*; note tracheids. × 333.

Fig. 9.—Vegetative mycelium and haustoria. *a*, haustorium showing suggestion of lobing; *b*, coiled haustorium; *c*, haustorium becoming vacuolate. × 400.

Fig. 10.—*a*, vegetative nuclei of the fungus showing nucleolus and polarization of the chromatin; *b*, host nucleus, showing clear region round the nucleolus. × 1,000.

Fig. 11.—Coiled haustorium and host nucleus, the latter shows the chromatin as irregular clumps. × 1,000.

Fig. 12.—Coiled haustoria in contact with the host nucleus. *a*, contraction of chromatin away from the nucleolus. × 1,000.

Fig. 13.—Host nucleus showing the chromatin in the reticulate condition, partly enwrapped by a haustorium. × 1,000.

"that it is certain that the plasma membrane of the host cell is not broken or pierced by the haustorium; it must be pushed in as the tip of the haustorium grows". Allen (1923) takes the same view in connection with the wheat rust; Robinson (1913) draws attention to the fact that many figures of past workers do not show clearly whether the haustoria in *Puccinia malvacearum* lie in the cell vacuole or in the cytoplasm. As the result of his investigations of this rust on *Althea rosea*, he described the hyphae as entering the cytoplasm and growing along strands to the nucleus. It is to be regretted that previous workers have used the term "cell membrane" in connection with this problem. Whether the primordial utricle is considered to be the "cell membrane" or whether the ectoplast is meant, is uncertain. If ectoplast is meant, it is immaterial whether it is broken or not, since the physical nature of protoplasm is such that an ectoplast will immediately form between the main body of the cytoplasm and a foreign object, in this case the haustorium. If the primordial utricle is meant a definite problem is involved. In *Uromycladium* the haustoria are in close contact with the cytoplasm of the host cell and in most cases cytoplasm can be detected around the infecting hypha. In a few cases this is doubtful, and the writer is inclined to believe that the haustoria sometimes penetrate the primordial utricle and enter the vacuole. Figures 15 and 16 are drawn from material which was slightly plasmolyzed in sugar solution and then fixed in Chromacetic. Figure 16 strongly suggests that the primordial utricle has been penetrated and that most of the haustorium lies in the vacuole. Figure 15 also shows that the haustoria may possibly enter the vacuole of the host cell. It is hoped at a later date to investigate this matter in more detail. As a rule, only one haustorium is found in a cell, although sections showing two or more entering from different sides are sometimes seen. In most cells, but not in all, the hyphae grow in the direction of the nucleus and may touch, or partly enfold, it. This may lead to distortion of the nuclear membrane, though lobed or greatly hypertrophied nuclei are very rare. The haustoria are simple in most cases, only occasional indication of lobing being seen (*a*, Fig. 9). They may be straight or coiled in varying degrees (see Figs. 9, 11 and 12). Throughout its life, the walls of the haustorium are thin and never show any thickening comparable to the sheath found in *Cronartium* (Colley, 1918). Frequently the haustoria are swollen at their base; this swelling commonly contains the nucleus (Figs. 12, 14 and 16). The cytoplasm of the haustorium is dense and finely granular at first, but later becomes vacuolate. The vacuoles at first appear some distance from the tip, leaving dense cytoplasm at the base and apex of the haustorium. Still later vacuolation occurs throughout the hypha, which begins to collapse, its function at this stage being, apparently, over. The nucleus of the haustorium varies little from the normal vegetative nucleus. It is more difficult to recognize a nucleolus and radiating chromatin material, but in favourable preparations this is possible. During the final vacuolation of the haustorium the nucleus tends to collapse and retains stains very tenaciously, making it impossible to get an accurate picture of the details at this stage. One other feature of interest in connection with haustoria is the position of the septum which cuts off the haustorial element. In some cases (*c*, Fig. 14) it is right at the base; here, however, it seems that the hypha is terminated by a haustorium. Where this organ is lateral there frequently appears to be no segmentation of a specialized cell, but merely an outgrowth which penetrates the host cell wall and later receives the nucleus of the mother cell (Figs. 12 and 11).

Apart from the stimulation already referred to (page 218), there is the effect of the parasite on the host nucleus. The normal nucleus in *Acacia stricta* contains a single nucleolus or rarely two nucleoli, and the chromatin material is in the form of a fine reticulum. Under the influence of the fungus the nucleus enlarges and the chromatin material becomes aggregated into irregular masses at points which probably represent the joins in the original reticulum (Figs. 14, 10, 16, 12, and 15, in order). The occurrence of several of the above stages in a uniformly stained preparation, indicates that the difference is not due to variations of technique. As the nucleus enlarges, the chromatin material appears to pull away from the nucleolus. In the earlier stages of the investigation it was thought

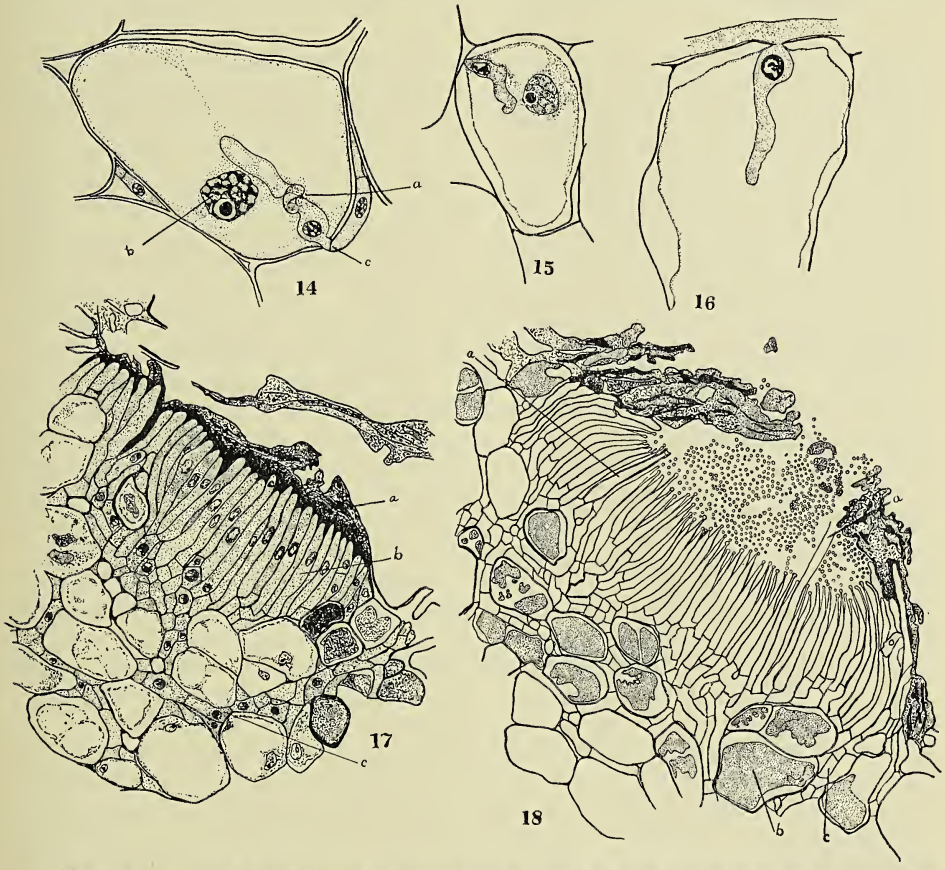

Fig. 14.—*a*, coiled haustorium becoming vacuolate; *b*, host nucleus showing clumping of the chromatin; *c*, wall separating haustorium from main mycelium. × 1,000.

Fig. 15.—From material plasmolysed in sugar solution and then fixed. Note contraction of the primordial utricle away from the wall except at the point of entry of the haustorium. × 1,000.

Fig. 16.—From the same material as fig. 15, note again the contraction of the primordial utricle away from the wall but not away from the haustorium. × 1,000.

Fig. 17.—Developing pycnium. *a*, crushed cells; *b*, young sporophores; *c*, contributing hyphae. × 533.

Fig. 18.—Adult pycnium. *a*, intrahymenial paraphyse; *b*, host cells containing plastic substances; *c*, contributing hyphae. × 390.

that this was due to faulty fixation, but variation of the fixative fails to produce a corresponding variation in the nuclear structure. Rice (1927) figures a similar condition in the host nucleus due to rust influence, but Allen (1923*b*), dealing with wheat infected with *Puccinia graminis tritici*, shows no indication of this phenomenon. In the wheat plant the nucleus does not possess such a marked reticulum, and the chromatin tends to collect on one side of the nucleus. Swelling of the nucleus in the acacia rust gall may cause an increase to twice the normal diameter. When vacuolation starts in the haustorium, the host nucleus usually begins to collapse (Fig. 15) and is more retentive of stains; this collapse continues and in the dead cells of the gall the nucleus is represented as an undifferentiated mass slightly larger than the unaffected nuclei.

The Pycnia.

As is usual in the rusts, the pycnia are the first spore-producing organs formed. In *Uromycladium Tepperianum* they are typically flask-shaped, and subcortical in origin. McAlpine (1906) states that the pycnia are produced under the cuticle, and Clements and Shears (1931), evidently following McAlpine, also describe them as subcuticular; this, however, is incorrect. The error could easily occur if the examinations were made of hand-sections, because the cells above the developing pycnia are greatly crushed and stain in some cases as a uniform mass resembling a very much thickened cuticle. In some cases the pycnia may underlie seven or eight layers of cells. There is a slight variation in size of the pycnia—the average being about 150μ in diameter. They are reddish-brown when young but soon appear blackish; in transmitted light they are brown. The hyphae which go to form the pycnia aggregate between the cells of the cortex and gradually push the host cells apart. In this mass of hyphae a pseudo-parenchymatous region is differentiated, which in its turn produces the young sporophores. The sporophores elongate parallel to each other and crush the cells external to the young pycnium (Fig. 17). A few of the outer cells remain sterile and form a very poorly defined band of periphyses, which are never sufficiently developed to project as hairs (Fig. 18). The sporophores then commence to bud off pycnospores and the pressure caused by the increasing mass of spores soon ruptures the overlying tissue and an ostiole is formed. This ostiolate and almost nonperiphysate pycnium may be compared with that of *Milesia marginalis* (Hunter, 1927), which lacks periphyses yet possesses a definite ostiole. The pycnia of *Uromycladium* are more indefinite than those of *Milesia*. There is a tendency, at times, for the outer tissue to be cast off, leaving an exposed hymenial layer. Lateral fusion of the pycnia sometimes occurs. At the base of the pseudo-parenchymatous layer are radiating cells which Colley (1918) in describing *Cronartium* has termed "contributing hyphae". These are in communication with the main mass of intercellular mycelium and serve as a means of transport for the food materials. In the young and even in the well-developed pycnia these hyphae have fairly dense cytoplasm and usually a number of small darkly-staining granules. Towards the end of the life of the pycnium vacuoles appear and increase in size, till eventually most of the basal hyphae are almost devoid of contents but for a small residuum of darkly staining protoplasm, probably representing the nucleus. At that stage the pycnia cease to produce spores.

The hymenial layer of the pycnium (Fig. 19) consists of parallel hyphae about 30μ to 40μ long and 2μ to 3μ in diameter. They are usually swollen slightly

in the centre and taper towards the apex. The nucleus is situated about the centre but moves somewhat during division. In the resting stage it varies little from the vegetative nucleus except in size, 3μ to 4μ by 3μ, as compared with 2μ to 3μ by 2μ in the vegetative hyphae, and chromatin content. In the vegetative nucleus the polarization of the chromatin is usually observable, but in the nuclei of the sporophores the polarization is not so definite and frequently difficult to demonstrate; the chromatin material is distributed round the nuclear membrane in the form of small irregular lumps. The nuclear membrane in very active nuclei is indistinct and, apparently, occasionally absent (cf. Blackman, 1904, fig. 49). A prominent nucleolus is invariably present. The cytoplasm of the sporophore is very finely granular, slightly vacuolate at the base and denser towards the apex.

The division of the sporophore nucleus has been examined with a fair degree of detail, principally from preparations stained with Iron Alum Haematoxylin, the observations, wherever possible, being checked by comparison with material stained in Flemming's Triple or Newton's Gentian Violet. Immediately prior to division of the nucleus the chromatin ceases to show even the polarization phenomenon recorded above and collects into irregular lumps scattered over the nuclear membrane. There may be as many as twenty of these chromatin masses, so they do not appear to bear any direct relationship to the chromosomes (1, Fig. 20). At this stage there is a slight indication that the centrosome has divided (see 2, Fig. 20), but this has not been definitely established. The nucleus then elongates and the membrane becomes indistinct (4, Fig. 20) and at the same time the chromatin material collects and forms a smaller number of deeply staining masses which are here interpreted as chromosomes (6, Fig. 20). There appear to be five or six of these. No definite metaphase condition, nor any evidence of a spindle, has been seen. Centrosomes are inconspicuous or absent, although some preparations (as in 9, Fig. 20) show bodies which may be interpreted as such. These, however, may only be chromosomes or portions of chromosomes slightly separated from the main group. Anaphase figures are the most frequent found in sections. Figure 20 (5) is difficult to interpret. It is probably an early anaphase, indicating a longitudinal division of the chromatin comparable to that figured by Blackman (1904, fig. 57) in *Gymnosporangium*. The process of separation of the chromosomes is irregular and individuals appear to lag behind (cf. 7, 8, 9, Fig. 20). In the majority of cases observed it is possible to distinguish some material joining the two groups of chromosomes towards the end of anaphase. This material is more retentive of stains than the general cytoplasm and appears to envelop the chromosomes. During these changes the dividing nucleus moves towards the apex of the sporophore. Telophase is indistinct. As the pycnospore nucleus commences to move into the pycnospore, traces of chromosome structure are still visible, but nearly all this detail becomes obscured when the nucleus passes through the slight constriction of the sporophore (10, Fig. 20), owing to the strong affinity of the chromatin for stains, at this stage. The sporophore nucleus begins to reorganize and continues to do so rapidly. If divisions are following each other quickly, the nuclear membrane may not be formed, and the next division commences before the spore is abstricted (6, Fig. 20). After reaching the apex of the sporophore the pycnospore nucleus contracts and stains less heavily. No nucleolus is discernible and the chromatin is scattered over the nuclear membrane (Fig. 21). The spores are freed partly

by abstriction and partly by septation. No indication of a collar such as Blackman described for *Gymnosporangium clavariaeforme* was seen, although numerous stains, including Congo Red, were used in an attempt to detect such a structure. The staining reactions shown by the upper end of the sporophore indicate that it differs from the lower part; saffranin readily stains the lower region, but not the apical part.

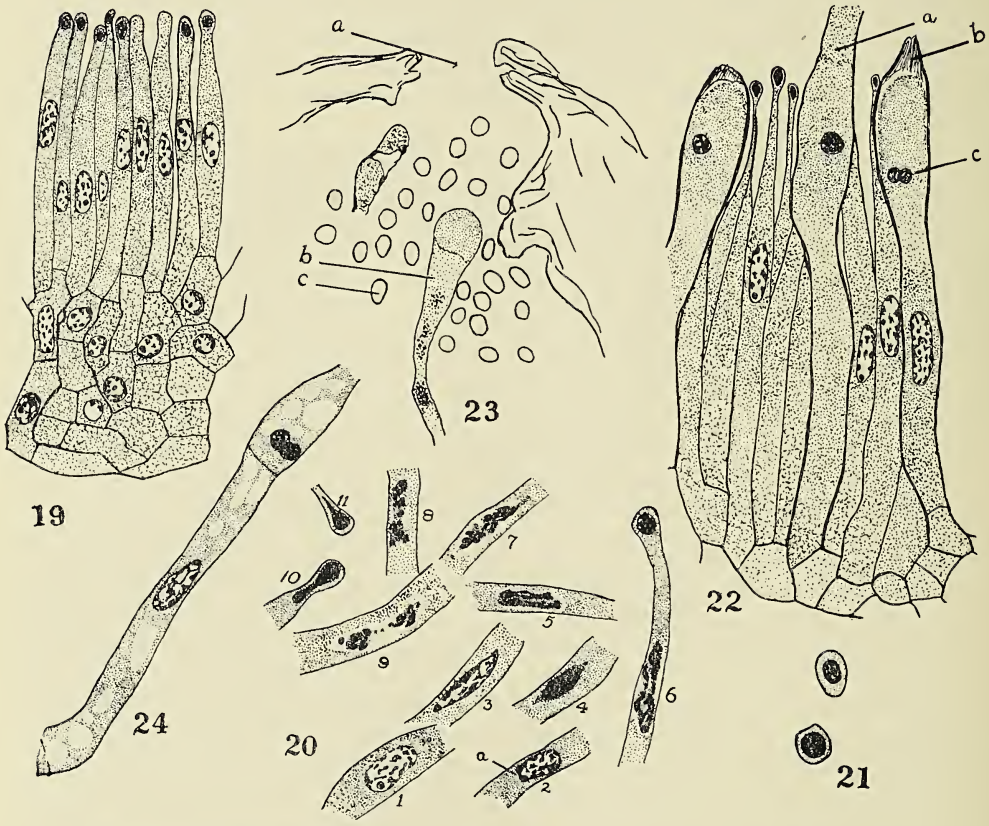

Fig. 19.—Hymenium of pycnium. × 1,500.

Fig. 20.—Nuclear division of the sporophore. 1-4, prophase; 5-9, anaphase; 10-11, telophase. At *a*, 2, note possible division of centrosome. × 1,500.

Fig. 21.—Pycnospores. × 1,500.

Fig. 22.—Hymenium of old pycnium. *a*, paraphyses; *b*, paraphyse wall cut obliquely; *c*, paired nuclei. × 1,500.

Fig. 23.—Mouth of pycnium showing apex of paraphyse. *a*, ostiole; *b*, apex of sporophore; *c*, pycnospore. × 1,500.

Fig. 24.—Paraphyse showing vacuolate condition of the cytoplasm and paired nuclei in the upper part, which is cut off by a septum. × 1,500.

Scattered throughout the hymenium are cells which are best termed intrahymenial paraphyses (Figs. 22, 23, and 24). These become evident in the mature pycnia. They arise in the position of sporophores, but differ from them in having a swollen apex which is usually vacuolated, and may be cut off from

the remainder by a septum (Fig. 24). When the pycnium is older these paraphyses grow out and some reach the mouth of the pycnium (Fig. 23). Colley (1918) mentions that in the pycnium of *Cronartium* hyphae grow out from the hymenium, but he does not figure, or discuss them. In *Uromycladium Tepperianum* they are a constant feature of the adult pycnium. In old pycnia binucleate examples of these paraphyses have been found and the possible significance of this will be discussed later. It was thought that these hyphae might secrete the pycnial fluid, but the evidence is against this view. The pycnial fluid is most abundant during the early life of the pycnia when these paraphyses are absent. They may function as dispersing organs, their upward growth forcing the spores out through the ostiole. A third suggestion is that they may be concerned with the formation of the binucleate stage in the life history of the fungus (cf. Craigie, 1933).

Acknowledgments.

The thanks of the writer are due to Mr. G. Samuel of South Australia, Mr. Geach of Canberra, and Mr. Cheel of Sydney, for supplying information regarding the collections in their charge and for permission to examine some of the specimens; to Mr. C. C. Brittlebank of Victoria for information bearing on the life of the galls; to Professor T. G. B. Osborn, Professor J. McLuckie, and Miss L. Fraser, M.Sc., of the Department of Botany, Sydney University, for suggestions and helpful criticism throughout the course of the work.

Summary.

1. The paper is the first of a series dealing with the Australian genus of gall-forming rusts, *Uromycladium*.

2. An evolutionary arrangement of the species is suggested.

3. The host range of *Uromycladium Tepperianum* is discussed, and possible physiological specialization indicated.

4. The genus *Uromycladium* is composed of microcyclic species.

5. The galls formed by *Uromycladium Tepperianum* on *Acacia stricta* are described in detail. Most of the gall is composed of xylem tissue which is unlignified.

6. It is suggested that the fungus utilizes the material which would normally produce lignification.

7. The galls are usually annual but may be perennial.

8. The vegetative mycelium is described, beginning at the stage when the gall is first discernible. The mycelium is localized at the seat of infection. The cells of the mycelium are uninucleate, the nuclei possessing a nucleolus, and a polarized chromatin network, possibly with a centrosome.

9. The haustoria may arise at any point along a hypha, and it is suggested that in some cases they may lie in the cell vacuole.

10. Changes in the host nucleus are described.

11. The pycnia are subcortical, non-periphysate, and imperfectly ostiolate. They may tend to become indefinite by lateral fusion.

12. Stages in the division of the pycnospore nucleus are recorded. Structures which were interpreted as chromosomes were seen. These are five or six in number.

Literature Cited.

ALLEN, RUTH, 1923.—A Cytological Study of Infection of Baart and Kanrad Wheat by *Puccinia graminis tritici*. *Journ. Agr. Res.*, 23, p. 571.

————, 1923*b*.—Cytological Studies of Baart, Kanrad and Mindum Wheats by *Puccinia graminis tritici* forms III and XIX. *Journ. Agr. Res.*, 26, p. 591.

ARTHUR, J. C., 1906.—Eine auf die Struktur und Entwicklungsgeschichte begrundete Klassification der Uredineen. *Result. Sci. Congr. Inter. Bot. Vienne*, 1905, pp. 331-348.

————, 1929.—The Plant Rusts. John Wiley and Sons, New York.

BLACKMAN, V. H., 1904.—On the Fertilisation, Alternation of Generations, and General Cytology of the Uredinae, *Ann. Bot.*, 18, p. 323.

BUTLER, E. J., 1930.—Some Aspects of the Morbid Anatomy of Plants. *Ann. App. Biol.*, 17, p. 175.

CLEMENTS and SHEARS, 1931.—The Genera of Fungi. Wilson and Co., New York.

COLLEY, R. H., 1918.—Parasitism, Morphology and General Cytology of *Cronartium ribicola*. *Journ. Agr. Res.*, 15, p. 619.

CRAIGIE, J. H., 1933.—Union of Pycnospores and haploid Hyphae in *Puccinia Helianthi* Schw. *Nature*, 1933, Jan. 7, p. 25.

DODGE, B. O., and ADAMS, J. F., 1918.—Some Observations on the Development of *Peridermium cerebrum*. *Mem. Torr. Bot. Club*, 17, p. 253-261.

GAUMANN, E. A., and DODGE, C. W., 1928.—Comparative Morphology of Fungi. McGraw Hill, New York.

HARSHBERGER, J. W., 1902.—Two Fungus Diseases of the White Cedar. *Proc. Acad. Sci. Nat. Phila.*, 1902, p. 461-504.

HUNTER, LILIAN, 1927.—Comparative Study of Spermagonia of Rusts of *Abies*. *Bot. Gaz.*, 83, p. 1.

LUDWIG, F., 1890.—Eine neue verheerende Rostkrankheit australischer Akazien, verursacht durch *Uromyces* (*Pileolaria*) *Tepperianus* Sacc. *Centr. f. Bakter. u. Parasit.*, Bd. vii, 583.

MAGNUS, P., 1892.—Zur Kenntniss des Verbreitung einiger Pilze. *Ber. Deutsch Bot. Gesell*, 10, p. 195.

————, 1900.—Studien an der endotrophen mycorrhiza von *Neottia nidus-avis*. *Jahrb. f. Wiss. Bot.*, Bd. xxiv, p. 213.

MCALPINE, D., 1905.—A New Genus of Uredineae—*Uromycladium*. *Ann. Mycol.*, 3, p. 303.

————, 1906.—The Rusts of Australia. Government Printer, Melbourne.

OLIVE, E. W., 1908.—Sexual Cell Fusion and Vegetative Nuclear Division in the Rusts. *Ann. Bot.*, xxii, p. 331.

RICE, MABEL A., 1927.—The Haustoria of Certain Rusts and the Relation Between Host and Pathogene. *Bull. Torr. Bot. Club*, 54, p. 63.

ROBINSON, W., 1913.—On some Relations between *Puccinia malvacearum* (Mont.) and the tissues of its host plant (*Althaea rosea*). *Mem. Proc. Manch. Phil. Soc.*, 1913, lviii, xi, p. 1.

SACCARDO, P. A., 1889.—Mycetes aliquot australiensis. *Hedw.*, 28, p. 126.

SAMUEL, G., 1924.—Some New Records of Fungi for South Australia. Part iii. *Trans. Roy. Soc. South Austr.*, xlviii, p. 149.

SANFORD, E., 1888.—Microscopical Anatomy of Gymnosporangium Galls. *Ann. Bot.*, i, pp. 263-268.

STEWART, A., 1915.—An anatomical Study of *Gymnosporangium* Galls. *Amer. Journ. Bot.*, 2, 402-447.

WENT, F. A. F. C., 1904.—Drulloten en versteende vruchten van de Cocoa in Suriname. *K. Akad. v. Wetenschappen. Amst.*, 2e. sectie, x, 3.

WOOD, J. G., 1932.—Xeromorphic Structure (Abstract Only). *Rept. Aust. N.Z. Assoc. Adv. Sci.*, Sydney, p. 369.

WORNLE, P., 1894.—Anatomische Untersuchung der durch *Gymnosporangium*. Arter Levorgeruferen Misstiloung. *Forst. Nat. Nat. Leitz.*, 3, 68-84.